Exhibit A

Klenow Fragment (3′ → 5′ exo⁻)

#212S	125 units	$44
#212L	625 units	$176

high concentration available upon request

- *Generates probes using random primers*
- *Specific Activity: 20,000 units/mg*
- *Dideoxy sequencing*
- *Supplied with 10X Reaction Buffer*

Description: Klenow Fragment (3′→ 5′ exo⁻) is a proteolytic product of DNA Polymerase I which retains polymerase activity, but has a mutation which abolishes the 3′→ 5′ exonuclease activity and has lost the 5′→ 3′ exonuclease (1).

Source: A genetic fusion of the *E. coli polA* gene, that has its 3′→ 5′ exonuclease domain genetically altered and 5′→ 3′ exonuclease domain replaced by maltose binding protein (MBP). Klenow Fragment exo⁻ is cleaved from the fusion and purified away from MBP.

Applications:
- Random priming labeling
- DNA sequencing by the Sanger dideoxy method (2)
- Second-strand cDNA synthesis
- Second strand synthesis in mutagenesis protocols (3).

Reaction Buffer: 1X *E. coli* Polymerase I/Klenow Buffer [10 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 7.5 mM dithiothreitol]. Supplement with dNTPs (not included). Klenow Fragment exo⁻ is also 50% active in all four standard NEBuffers when supplemented with dNTPs.

Notes on Use: Heat inactivated by incubating at 75°C for 20 minutes.

When using Klenow Fragment (3′→ 5′ exo⁻) for sequencing DNA using the dideoxy method of Sanger et al., an enzyme concentration of 1 unit/5 µl is recommended.

Quality Assurance: Purified free of endonuclease and contaminating exonuclease. Each lot is functionally tested in dideoxy sequencing protocols and in random priming reactions.

Unit Definition: One unit is defined as the amount of enzyme required to convert 10 nmoles of dNTPs to an acid-insoluble form in 30 minutes at 37°C.

Unit Assay Conditions: 40 mM KPO_4 (pH 7.5), 6.6 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 20 µM dAT copolymer, 33 µM dATP and 33 µM ^{3}H-dTTP.

Concentration: 5,000 units/ml.

Storage Conditions: 0.1 M KPO_4 (pH 7.5), 1 mM dithiothreitol, and 50% glycerol. Store at –20°C.

References:
(1) Derbyshire, V. et al. (1988) *Science* 240, 199–201.
(2) Sanger, F. et al. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
(3) Gubler, U. (1987) *Methods Enzymol.* 152, 330-335.

T4 DNA Polymerase RH

#203S	150 units	$50
#203L	750 units	$200

- *Isolated from a recombinant source*
- *Removal of 3′ overhangs*
- *Fill-in of 5′ overhangs*
- *Second strand synthesis in site-directed mutagenesis*
- *Supplied with 10X Reaction Buffer*

Description: T4 DNA Polymerase catalyzes the synthesis of DNA in the 5′→ 3′ direction and requires the presence of template and primer. This enzyme has a 3′→ 5′ exonuclease activity which is much more active than that found in DNA Polymerase I. Unlike *E. coli* DNA Polymerase I, T4 DNA Polymerase does not have a 5′→ 3′ exonuclease function.

Source: Purified from a strain of *E. coli* that carries a T4 DNA Polymerase overproducing plasmid.

Applications:
- 3′ overhang removal to form blunt ends (1,2).
- 5′ overhang fill-in to form blunt ends (1,2).
- Single strand deletion subcloning (3).
- Second strand synthesis in site-directed mutagenesis (4).
- Probe labeling using replacement synthesis (1,2).

Reaction Buffer: 1X T4 DNA Polymerase Buffer [50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.9 @ 25°C)]. Supplement with 50 µg/ml BSA and dNTPs* (not included in supplied 10X buffer). Incubate at temperature suggested for specific protocol.

* Refer to specific protocol to determine required dNTP concentrations.

Blunt ending 3′ and 5′ overhangs: We recommend using 100 µM of each dNTP, 1–3 units polymerase/µg DNA and incubation at 12°C for 20 minutes in the above reaction buffer (1,2).

Notes on Use: Heat inactivated by incubating at 75°C for 10 minutes.

T4 DNA Polymerase is active in all four standard NEBuffers when supplemented with dNTPs.

Quality Assurance: Purified free of endonuclease.

Unit Definition: One unit is the amount of enzyme which incorporates 10 nmoles of dNTP into acid precipitable material in 30 minutes at 37°C (5).

Unit Assay Conditions: 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.9 @ 25°C), 33 µM dATP, dCTP and dGTP, 33 µM ^{3}H dTTP, 70 µg/ml denatured calf thymus DNA, and 170 µg/ml BSA. Note: These are not suggested reaction conditions; refer to Reaction Buffer.

Concentration: 3,000 units/ml.

Storage Conditions: 100 mM KPO_4 (pH 6.5), 10 mM 2-mercaptoethanol and 50% glycerol. Store at –20°C.

References:
(1) Tabor, S. and Struhl, K. (1989) in *Current Protocols in Molecular Biology* (Ausubel, F.M., et al., eds) pp. 3.5.10-3.5.12, John Wiley & Sons, New York.
(2) Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, second edition, pp. 5.44-5.47, Cold Spring Harbor Laboratory, Cold Spring Harbor.
(3) Dale, R., McClure, B. and Houchins, J. (1985) *Plasmid* 13, 31-40.
(4) Kunkel, T.A., Roberts, J.D. and Zakour, R.A. (1987) *Methods Enzymol.* 154, 367-382.
(5) Panet, A., van de Sande, J.H., Loewen, P.C. and Khorana, H.G. (1973) *Biochemistry* 12, 5045–5050.

Cloned at NEBiolabs RH Recombinant Enzyme